serum was described by Ruczkowska.⁴⁰ This method has a good specificity but Wilkinson⁴¹ found it less sensitive than the F.T.A.-A.B.S. test.

By the use of conjugated antisera specific for the various classes of immunoglobulins it has been shown that the major antibody detected in the F.T.A.-A.B.S. test is IgG and that IgM and IgA play a subsidiary part.^{42 43} There is some evidence that antibody formed early in infection is IgM and that the test can be made even more sensitive in early syphilis if a conjugate specific for IgM is used. This procedure has also been found useful in the differentiation of congenital syphilis in the newborn baby from passive transfer of maternal antibody across the placenta. Since IgM does not normally cross the intact placental barrier, demonstration of specific IgM antitreponemal antibody in the child's circulation is presumptive evidence that it is infected—particularly if there is a rise in titre.^{44 45}

Staining by immunofluorescence methods has also been used to detect *T. pallidum* in material from early lesions. Both direct and indirect methods can be used, the reagents being absorbed with Reiter treponemes to remove group reactive antibody. The results seem as reliable as conventional examination by dark-ground microscopy.⁴⁶⁻⁴⁸

Treponemal Haemagglutination (T.P.H.A.) Test

In this test, described by Tomizawa and Kasamatsu⁴⁹ and based on work by Rathlev,⁵⁰ the antigen is a suspension of formalized tanned sheep red cells sensitized with an extract of *T. pallidum*. Cross-reacting antibodies are absorbed from sera before testing. It has not yet been fully evaluated, but preliminary reports suggest that it is both specific and sensitive. Unlike the T.P.I. and F.T.A.-A.B.S. tests it can easily be performed quantitatively and is technically much easier to set up than either of these tests.

The relative sensitivity of the various tests in untreated syphilis is compared in the Table.

Response of Specific Tests to Treatment

Unless treatment is first given during the primary or secondary stage of syphilis, both the T.P.I. and the F.T.A.-A.B.S. tests usually remain positive for many years, often for the patient's lifetime. In a minority of patients treated late in the disease the T.P.I. test may become negative while the F.T.A.-A.B.S. test

is still positive. Almost all patients treated for primary or secondary syphilis show a slow reversal of the T.P.I. test to negativity; the F.T.A.-A.B.S. test seems even slower to reverse. The behaviour of the T.P.H.A. test over a period of time is not yet certain.

The persistence of reactivity in these specific tests makes them of little or no value in the assessment of progress after treatment. Whether persistence of specific antibody is a reflection of persisting *T. pallidum* in the host is still unresolved. The value of these tests lies their use in the differentiation of positive tests for reagin due to latent treponemal disease from those due to biological false-positive reactions and in the diagnosis of late manifestations of syphilis when the tests for reagin are negative or inconclusive. A positive result in the T.P.I. or F.T.A.-A.B.S. tests is an indication of treponemal infection past or present; it does not necessarily mean that the disease process is active. Indeed, the tests for reagin may offer a better guide to activity; this may be suspected if the V.D.R.L. test is reactive at a dilution of 16 or above, particularly if there is a rising titre. No test at present available will distinguish syphilis from yaws, pinta, or endemic non-venereal syphilis.

Relative Sensitivity of Tests in Untreated Syphilis

Test	Stage of Syphilis			
	Primary	Secondary	Latent	Late
V.D.R.L., A.R.T.	..	+++	++	++
R.P.C.F.T.	..	+++	++	++
T.P.I.	..	+++	+++	+++
F.T.A.-A.B.S.	..	+++	+++	+++
T.P.H.A.*	..	+++	+++	+++

*Based on early reports.

Chlamydia and Non-Specific Urethritis

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Ever since Neisser⁹⁰ described the gonococcus in discharge from the eye and from the genital tract the history of urethritis has been linked with that of conjunctivitis.^{91 92} Organisms that infect the genital mucous membranes also attack the conjunctiva, where their effects may be studied more readily than in the

genital tract. Until Chlamydia was cultured, 50 years after the agent was first described in smears from the eye⁹³ and then from the genital tract, its significance remained controversial despite many studies.⁹⁴ T'ang *et al.*⁹⁵ cultured the causative agent of trachoma in the yolk sacs of fertile hen's eggs. The conjunctiva of volunteers was inoculated with the agent and trachoma resulted.^{96 97} Chlamydia was isolated from the eye of a baby suffering from ophthalmia neonatorum and from the cervix of the mother of such a baby by Jones *et al.*⁹⁸ It was also recovered from the cervix of a mother of twins suffering from ophthalmia neonatorum; this mother was suffering from trachoma and one of the twins developed that condition.⁹⁹ Jones and Collier^{100 101} produced trachoma by inoculating the conjunctiva of an adult with agent from the eye of a baby suffering from ophthalmia neonatorum; this agent was of genital

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origin because the baby had acquired the infection from its mother's genital tract; another inoculation of this volunteer with an isolate from the cervix of the mother of such a baby produced inclusion conjunctivitis only. Chlamydia was isolated from babies suffering from ophthalmia neonatorum, from their mothers who were suffering from genital infection, and from their fathers who were suffering from "non-specific" urethritis (NSU).^{102 103}

Chlamydia was isolated from two of nine unselected men who had presented because of NSU.¹⁰⁴ The isolate from the urethra of an additional such patient was used to inoculate the conjunctiva of three baboons and produced conjunctivitis typical of TRIC agent in all. Subsequently chlamydia was isolated from up to 21% of men who presented with NSU and up to 35% of the female contacts of such men; also chlamydia agent was isolated from the genital tract in the cases of men and women presenting because of fresh infection of the eye by TRIC agent, and from their sexual contacts.¹⁰³

Terminology

Because an isolate from a patient suffering from trachoma was indistinguishable from an isolate from a baby suffering from ophthalmia neonatorum, even by inoculation of volunteers,^{100 105} such an isolate was named TRIC agent, the TR- standing for trachoma and the -IC for inclusion conjunctivitis.¹⁰⁶

The term "agent" is now used because members of the Chlamydia group are neither viruses nor bacteria: thus they have a characteristic development cycle; contain both DNA and RNA; possess cell and enzyme systems; and are inhibited in varying degrees by sulphonamides and tetracycline;¹⁰⁷ they are also inhibited by rifampicin, erythromycin, and trimethoprim¹⁰⁸ and to some degree by penicillin and by chloramphenicol.⁹⁵ This group of agents has been called Bedsonia (after the late Sir Samuel Bedson, who isolated and characterized the agent of psittacosis), and by many different names which has resulted in increasing confusion. Applying the taxonomic rules of nomenclature, Page¹⁰⁹ re-proposed the name Chlamydia for the genus. The term TRIC agent is suitable for a chlamydial ocular pathogen that is isolated from characteristic disease of the eye; but an isolate from an extraocular site should be designated only by the genus name (Chlamydia) unless there is evidence that it is TRIC agent or lymphogranuloma venereum (LGV) or another chlamydial agent. The limitations of the micro-immunofluorescence test (see below) are not yet known, but probably it may be able to identify an agent as one of the different serotypes of TRIC agent or of LGV, or as another member of the Chlamydia. Nevertheless, it is not yet known whether there may be variation in pathogenicity (to the eye or to other tissues) among the individual members of each serotype, among the different serotypes of TRIC agent, or among the serotypes of LGV.

New Tests for Chlamydial Infection

ISOLATION IN CELL CULTURE

The fact that Chlamydia was isolated in yolk sac from the cervix in only a quarter of 28 mothers of babies suffering from TRIC ophthalmia neonatorum¹⁰³ indicated the need for more sensitive methods.

The technique of culture in irradiated McCoy-cells^{110 111} provided the first alternative to culture in yolk sac for the primary isolation of Chlamydia. Comparisons have shown that this technique is appreciably more sensitive for detecting Chlamydia in clinical specimens than the detection of inclusions and isolation in yolk sac.¹¹²⁻¹¹⁴ Using a shorter simplified form of this method¹¹⁵ isolates were obtained from 18 of 41 (44%) of men who had presented because of NSU and from five of 21 of their female sexual contacts.¹³¹ The sensitivity of the technique and the resistance of the McCoy-cell culture to bacterial contamination permitted the culture of chlamydia from the rectum,^{112 116-8} whereas culture from that site had previously been unsuccessful. A carefully devised system of controls

showed that cross-contamination did not occur in the laboratory using this method.^{112 113 118}

The technique of culture in irradiated McCoy-cells has reached a level of sensitivity and practicability¹¹⁹ that permits it to be used generally for diagnosis, epidemiological studies, and assessment of the results of treatment.

MICRO-IMMUNOFLUORESCENCE TEST

Isolates of chlamydia may be divided into subgroup A, whose inclusions contain glycogen and hence stain with iodine (this subgroup includes TRIC agent and most isolates of LGV), and subgroup B, whose inclusions do not contain glycogen and hence do not stain with iodine; this subgroup is mainly of avian and animal origin.¹²⁰ The distinction between TRIC agent and LGV agent in the laboratory has, until recently, depended on the somewhat irregular patterns of pathogenicity to animals of the two agents; hence the labelling of an isolate as LGV rather than as TRIC has been made primarily on its clinical origin.¹²¹

The micro-immunofluorescence (micro-IF) test¹²² has proved to be the best method of immunological classification.

So far it has shown the existence of seven main serotypes of TRIC agent and three serotypes of LGV agent.^{121 123-6} Serotypes A, B, and C were isolated from patients suffering from endemic trachoma in the Middle East, Africa, and Asia, where eye-to-eye transmission is the presumed method of spread of infection. In our laboratory only two such isolates were type D.¹²⁷ With few exceptions serotypes D, E, F, and G were isolated from the eye, rectum, or the genital tract of patients seen in London, Copenhagen, and parts of the U.S.A. where the presumed route of transmission is genital.

The precision of the micro-IF test makes it a valuable immunological method for studying infection by Chlamydia: thus it can be used to distinguish TRIC agent from LGV agent and to identify serotypes of Chlamydia for the preparation of intradermal tests; this may prove useful because the Frei intradermal test and the so-called specific acid-extracts for intradermal tests have given some positive but confusing results in general studies of chlamydial infection.¹²⁸

Measurement of antichlamydial group antibody by a complement-fixation test (LGVCF) using chlamydial group antigen is insensitive and of limited use.¹²⁸ The micro-IF test is more sensitive; moreover, it detects antibodies to individual chlamydial serotypes. Nevertheless, the results must be interpreted with caution until the range of subgroup B Chlamydia definable by the micro-IF test has been worked out, and representative subgroup B serotypes have been included in the test.

PATHOGENICITY OF CHLAMYDIA

Chlamydia is a pathogen in the eye, and it is likely also to be a pathogen in the genital tract and the rectum because Chlamydia from the genital tract produces disease of the eye, as shown by each baby suffering from TRIC ophthalmia neonatorum, and by the inoculation of the conjunctiva of man^{100 101} and of experimental animals.^{104 118} An isolate from the rectum of the mother of a baby suffering from TRIC ophthalmia neonatorum produced typical follicular conjunctivitis in two baboons and, established urethral infection in a male baboon, from which the agent was re-isolated.¹¹⁸ The association of the presence of Chlamydia in the genital tract with inflammatory change is close, for example in the parents of babies suffering from TRIC ophthalmia neonatorum, and in adults presenting with fresh ocular infection by TRIC agent; moreover, the inflammatory changes associated with the presence of TRIC agent may be found in the sexual partners of such patients.¹⁰³ For the rectum,¹¹⁷ as for other sites, there is an association between the

presence of inflammatory changes and the isolation of Chlamydia. Furthermore, the clinical signs associated with the presence of Chlamydia in the conjunctiva, genital tract, and rectum may closely resemble each other.

In early studies large amounts of the urethral epithelial cells to be tested were obtained by a curette.¹⁰²⁻¹⁰⁴ This made the study of a control group impracticable. A miniature endo-urethral swab has been devised¹²⁹ which can be passed to the bulb of the urethra in men, and results using this atraumatic method to collect material for culture in irradiated McCoy-cells are as good as those using a urethral curette. This method will permit the study of a control group of men and repeated tests of the urethra to assess the results of treatment of chlamydial urethritis in male patients.

Genital Reservoir of Chlamydial Infection

Non-gonococcal urethritis (NGU) is becoming increasingly common. In England and Wales in 1970, 48,550 cases of NGU were reported in men, compared with 54,717 cases of gonorrhoea in men, women, and children.⁵⁷⁻⁵⁸ If the equivalent infections in women are considered probably the incidence of non-gonococcal genital infection in men and women must be even greater than that of gonorrhoea. About 90% of NGU in men is NSU. In a joint study, carried out at the Whitechapel Clinic of The London Hospital and the Institute of Ophthalmology, eight

cases of gonococcal ophthalmia neonatorum were seen over a period in which 44 cases of ophthalmia neonatorum due to TRIC agent were seen. Factors concerned in this ratio include the incidence of each infection in the community, the relative clinical silence of genital infection due to TRIC agent (so that parents tend to remain untreated), and the longer incubation of ophthalmia neonatorum due to TRIC agent than that due to gonorrhoea.

Hence a considerable proportion of "non-specific" genital infection is related to Chlamydia. The new developments will enable us to begin to define how big this proportion is; to study control groups; to determine if any of the Chlamydia are non-pathogenic in the eye, genital tract, or elsewhere; and to study further special forms of disease such as Reiter's disease, abacterial pyuria, proctitis, and salpingitis. Thus there is evidence already that salpingitis commonly occurs in the mothers of babies suffering from ophthalmia neonatorum due to TRIC agent.¹⁰³⁻¹³⁰ The culture of specimens obtained at laparoscopy should show whether Chlamydia is present in the affected fallopian tubes or not. Such studies will lay the foundations for defined assessment of treatment for disease due to Chlamydia.

It would be surprising if there were no other agent concerned as a cause of NSU; therefore it will be of particular interest to test for other agents those patients in whom efficient tests (cell culture and immunological studies) for Chlamydia have given negative results.

Persistence of Treponemes after Treatment

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Treponeme-like forms have been found by workers in France, the United States of America, Italy, and Britain in material from patients suffering from late syphilis, even after large amounts of antisypilitic treatment; they have also been found after the treatment of early syphilis. The organisms have been recovered from lymph nodes, aqueous humour, cerebrospinal fluid, brain, arteries affected by temporal arteritis, and bone. The subject has been reviewed.⁵¹⁻⁵⁶

Syphilis in England and Wales

Syphilis is a very well-controlled disease here,⁵⁷⁻⁵⁸ unlike gonorrhoea and non-gonococcal genital infection; thus the number of reported cases of infectious syphilis was only 1,628 in 1970, compared with the 4,986 cases in 1939. Nevertheless, in the 25 years starting in 1946 over 178,000 cases of acquired and congenital syphilis were reported. If the number of persons treated during the war is added it is clear that there are many people in this country who know that they have received treatment for syphilis. If unnecessary doubts are raised now about the clinical effectiveness of treatment this will cause ill-founded anxiety, fear, and the reopening of long-closed problems for many people.

Are they *Treponema pallidum*?

The fundamental question is whether treponeme-like forms found after the treatment of syphilis are *Treponema pallidum*. If they are, they may be virulent, avirulent, or of low virulence. Alternatively, these forms may be artefacts, natural filaments, or other treponemes. Thus Wilkinson⁵⁹ reported that glass filaments could resemble treponemes, while Montenegro *et al.*⁶⁰ concluded that some structures previously reported as treponemes by their group were artefacts but that others were "incontrovertible" treponemes.

Treponeme-like forms may be seen on microscopical examination of a specimen from a patient or from an animal that has been inoculated with such a specimen. They may be seen in wet preparations by darkfield microscopy, which allows their characteristic motility to be recognized, or in fixed material which may be stained with silver or by a fluorescent antibody method. Experienced observers who have looked for "persistent treponemes" have reported the presence of structures morphologically typical of *T. pallidum* in fixed preparations. Nevertheless, if other structures can closely resemble *T. pallidum*, conceivably some may be indistinguishable on morphological grounds. But artefacts could not show true motility in wet preparations. Motile treponeme-like forms have been seen by different observers in aqueous humour and in cerebrospinal fluid. Thus Wilkinson⁶¹ found such forms in wet darkfield preparations of spinal fluid in six cases; in three the forms were motile but, perhaps not unexpectedly, did not show the vigorous movement that is shown by treponemes freshly obtained from the lesions of early syphilis.

The presence of virulent *T. pallidum* may be shown by the experimental infection of animals. The importance of a typical

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